SECONDARY PRODUCTIVITY OF TERRESTRIAL ECOSYSTEMS
ED. K. PETRUSEWICZ

WARSZAWA 1967 KRAKÓW

D. R. GIFFORD

Department of Forestry
and Natural Resources,
University of Edinburgh,
Scotland

3.3.4 An attempt to use ^{14}C as a tracer in a Scots pine (*Pinus silvestris* L.) litter decomposition study

1. INTRODUCTION

This paper describes an experiment to test the feasibility of using ^{14}C as a tracer for making observations on the decomposition of Scots pine (*Pinus silvestris* L.) litter. There were two objectives, to investigate the levels of uptake required to allow useful observations to be made on the microarthropods, and to undertake series sampling of the latter to determine the phenology of their contribution to the decay process, by detection of the ^{14}C levels in particular species.

^{14}C has been used by Grossbard (1963) to trace cellulose decomposition by the microflora, but attempts to use tracers in studies of the microarthropods associated with decomposition have been limited to work on the passage of ^{137}Cs through litter fauna (Reichle and Crossley 1965).

It is easy to see that decay processes in pine litter become increasingly advanced with increasing depth in the profile of the organic soil; numerous observations of the vertical distribution of microarthropods from widely differing habitats are available in the literature. But a number of factors besides their dependence on particular food substances influence the biology of these animals in decomposing litter; climate, the distribution of the microflora, pore space, the water regime are obvious examples, and it is not easy to draw conclusions concerning the feeding activity of particular microarthropods from simple observations of vertical distribution.

Use of a tracer to exhibit feeding relationships in such a system depends on the availability of a radionuclide of extensive half-life and, in the circumstances of this study, low health hazard. ^{14}C is suitable on both counts, and it is possible to introduce it into the decomposition cycle by incorporating it into the litter which forms the basic energy source for the soil animals.

2. METHODS

2.1 GENERAL

The fieldwork is being carried out in the Black Wood of Rannoch, Perthshire, in a dense pure stand of 75 year Scots pine, under which there is very sparse ground vegetation. The leaf fall is greatest in late summer, and in October 1964 two patches of litter labelled with ^{14}C and approximately 0.5 m square were put on the surface of the forest floor, after removal of the recent natural leaf fall. The tagged litter was covered with heavy plastic net of 0.3 cm mesh. Subsequent recovery and evaluation of the samples is described below.

2.2 PRODUCTION OF ^{14}C LABELLED LITTER

Scots pine seedlings were grown in a perspex chamber in a closed atmospheric system, in which the CO_2 had been replaced by $^{14}CO_2$ at the period of elongation of the young shoots. The system adopted was based on Jenkinson (1960) who grew ryegrass in a ^{14}C atmosphere, except that it was necessary to use natural light. This introduced many complexities into the design of the chamber, all stemming from the large radiation income and the consequent need to avoid extreme humidities and excess condensation, particularly on the walls and roof, but also on the plants themselves.

Temperature fluctuations inside the chamber were damped by introducing a cooling coil through which ethanol was pumped from a deepfreeze, and by fan circulation of the enclosed air over the coil. The pump and fan were both thermostatically controlled. The skin temperature of the chamber was stabilized at 27°C by pumping a 1% solution of formaldehyde through a perspex maze on the exterior of the chamber. Condensation was thus induced on the cooled coil, and by manipulation of the temperatures of the surfaces a coarse control of humidity was established. This prevented much fungal growth, which had marred early attempts to grow the seedlings in the chamber. The plants, which were two years old and approximately 15 cm high were rooted in coarse sand and fed with the nutrient described by Hewitt (1952) once daily.

The $^{14}CO_2$ was generated from $Na_2{}^{14}CO_3$ with HCl, and introduced into the chamber by partial evacuation. The chamber was previously cleared of natural CO_2 by passing the air through strong KOH solution and washing it, before introduction of the labelled $^{14}CO_2$. The plumbing was modified from that of Jenkinson (1960). For safety the chamber was run at negative pressure and housed in a greenhouse on the roof of the laboratory.

Two crops of seedlings were grown, each derived from approximately 100 plants and each incorporating 10 mC of ^{14}C.

On completion of the growth phase in late autumn the plants were dried off and the litter of the current year's growth retained for the experiments.

2.3 SAMPLE COLLECTION

A circular core cutter was used to sample in the area covered with labelled litter. The fauna of each core was extracted in a Macfadyen type high gradient extractor, and the core was then divided into 1 cm thick slices from the surface downward. These were dried at 105°C and used to determine the gross spread of ^{14}C down the profile. The animals were variously treated to determine their radioactivity.

2.4 PREPARATION OF SAMPLES FOR COUNTING

The low activity of the samples and the weak beta emission of ^{14}C make it simplest to use liquid scintillation counting after oxidation of the sample to CO_2 and absorption of the gas in a suitable base. The measurements reported here were derived by sample combustion in O_2, and absorption of the CO_2 in hyamine hydroxide.

Kelly and others (1961) described a very simple system of combustion in O_2 in glass with introduction of the absorbant into the flask when oxidation is complete. Kalberer and Rutschmann (1961) described combustion of the sample in a glass flask filled with O_2 with a supercooled extension containing the absorption medium, allowing the system to be completely closed prior to ignition. They advocated maximum sample weight of 0.25 g for a one litre flask of O_2 at atmospheric pressure, and arranged for the eventual removal of the dry-ice acetone mixture cooling the absorption medium once the flask temperature was low after combustion. Dobbs (1963) also outlined a glass flask system for combustion of samples in oxygen at atmospheric pressure, in which he inserted the premixed scintillator and absorption medium into the ignition flask after combustion, through a serum cap, using a hypodermic syringe.

Sheppard and Rodegker (1962) and Macfarlane and Murray (1963) each described sample combustion in O_2 at high pressure in a bomb. The system has obvious advantages in ensuring complete combustion of relatively large samples in a small vessel, as well as being safer than glass, but control gear is elaborate, particularly in Macfarlane and Murray's (1963) system, and there is no gain in simplification in handling the radioactive gas after combustion.

Choice of a suitable absorption medium is governed by its solubility as carbonate in the scintillator, and by its quenching properties. Hyamine hydroxide has been widely used, but ethanolamine (Kalberer and Rutschmann 1961) and phenethylamine (Dobbs 1963) are examples of weaker bases used successfully. The quenching effect of hyamine in this series of samples has not yet been adequately measured, but since all the material was converted to the same form for counting, comparability is not affected. Scintillators and solvents have been thoroughly reviewed by Rapkin (1964).

The system adopted for my experiment was to suspend the sample in a stainless steel cup in a glass reaction vessel which was then thoroughly flushed with O_2. 3 ml of hyamine hydroxide, 1.0 molar solution in methanol, was run in during the flushing. The vessel was sealed and the sample ignited by passing a 6 volt current through fine wire over the cup. A cotton train was used between the wire and the sample. 20 minutes was allowed for absorption and then two aliquots of 1 ml hyamine were taken from the chamber and mixed with NE213 scintillator (Nuclear Enterprises Ltd.) and counted.

Samples of pine needles were ignited whole, while litter in various stages of decomposition was ground in a pestle and mortar prior to combustion. Large animals, e.g. millipedes, were also ignited whole, but the microarthropods were bulked in cellulise wadding to avoid dispersal during combustion. Such dispersal is likely to lead to ignition of the methanol solvent in the absorbent, with consequent explosion of the system. Some system for separation [e.g. Dobbs (1963)] or supercooling (Kalberer and Rutschmann 1961) is essential for safety, but the vessel used in my experiments was inadequate in this respect and frequently ignited the absorption medium.

2.5 COUNTING PROCEDURE

The instrument used for counting was an I.D.L. liquid scintillation coincidence counter. The relatively high background, over 1 c.p.s. (count per second), made it necessary to count microarthropods in batches and all individuals of a particular species were bulked for ignition. Counting efficiency was 79% using an external standard, but quenching effects produced an undetermined reduction from this figure in the samples.

3. RESULTS

Activities are expressed as counts per second per mg dry weight. In this form direct comparisons are valid and significance of radioactivity can be determined by comparison with background counts, but specific activity cannot be quoted for any of the samples.

The needle litter derived from the tagged pine seedlings showed a count of 44.9 c.p.s./mg before initial decomposition. Approximately 100 g dry litter was used on each of the 0.5 m square plots.

Samples were collected after two months, six months and twenty months; in the two early sets no movement of radioactivity from the deposited leaf litter was detected, but this may have been due to faulty technique. The gross spread of ^{14}C into the system was investigated by sampling separate 1 cm strata, with the results given in Table I.

The twenty month series was carried down to 7 cm, but no activity was found below 3 cm. In addition a sparse growth of *Deschampsia flexuosa* (L.) Beauv. growing on the plots was tested and was found to be not radioactive.

As many species of microarthropods as possible were tested for radioactivity after the 20 month extraction. As might be expected, only the larger and more numerous species proved to have measurable amounts, and some of these had curiously high concentrations of activity. Table II lists the significantly radioactive species measured.

4. DISCUSSION

The rapid elimination of much of the radioactivity from the profile is a feature of the result in Table I. A large part of this must be due to respiratory activity of the microflora and fauna, but measurements of this have yet to be made on our plots. It is also clear that there is very little translocation of insoluble material during decomposition, at least in the early stages. No measurements were made in the deposition zone of the humus-iron podzol lying below the plots.

With the levels of activity used in this experiment the only individual animals in which radioactivity can be measured are large ones such as millipedes. The largest microarthropods were not sufficiently labelled for measurement by the techniques used here, while in small species insufficient biomass could be gathered to give significant readings.

However, there is a clear demonstration of radioactivity in a number of *Oribatei* and weakly significant activity in the gamasid predators. What is not clear is how much of this is due to contamination from the substrate, or how much is derived from included gut contents, containing ^{14}C material.

The animals have so far been collected dead, and experiments to determine feeding rate and assimilation using ^{14}C materials have not yet been begun. Until further data are collected it is not possible to indicate the source of the radioactivity in the microarthropods. Much is likely to be the result of fungal feeding; but a preliminary attempt to assess the radioactivity of the fungi by autoradiography was not successful, due to faulty technique.

It is evident that more concentrated work is required on the upper 2 cm of the profile, with stronger labelling, before either of our objectives can be reached but neither seems unattainable.

Thanks are due to H. M. Forestry Commission for providing the site on which this work is done, as well as a travel grant; to the Science Research Council for an equipment grant; to P. Tothill and J. D. Simpson, of the Department of Medial Physics, University of Edinburgh for much advice and to my colleague A. J. Hayes for useful discussions.

REFERENCES

Dobbs, H. E. 1963 — Oxygen flask method for the assay of tritium, carbon-14, and sulfur-35 labelled compounds — Analyt. Chem. 35: 783—786.

Grossbard, E. 1963 — A method of observing the incorporation of ^{14}C into microbial tissues during cellulose decomposition in the soil — Abstr. 8th int. Congr. Microbiol. Montreal, 1962. 63 pp.

Hewitt, E. J. 1952 — Sand and water culture methods used in the study of plant nutrition — Commonw. Bur. Hort. Plantat. Crops, Tech. Commun. No. 22. 1—548.

Jenkinson, D. S. 1960 — The production of ryegrass labelled with carbon-14 — Plant and Soil, 13: 279—290.

Kalberer, F., Rutschmann, J. 1961 — A rapid method for the determination of tritium, radiocarbon and radiosulphur in any organic test material by liquid scintillation counting — Helv. chim. acta, 44: 1956—1966 (Read in translation by E. F. Towndrow, A.E.R.E. Translation 914).

Kelly, R. G., Peets, E. A., Gordon, S., Buyske, D. A. 1961 — Determination of ^{14}C in biological samples by Schöniger combustion and liquid scintillation techniques — Analyt. Biochem. 2: 267—273.

Macfarlane, A. S., Murray, K. 1963 — ^{14}C and ^{3}H specific activities by bomb combustion and scintillation counting — Analyt. Biochem. 5: 284—287.

Rapkin, E. 1964 — Liquid scintillation counting 1957—1963: a review — Int. J. appl. Rad. Isotopes, 15: 69—87.

Reichle, D. E., Crossley, D. A. 1965 — Radiocesium dispersion in a cryptozoan food web — Hlth Phys. 11: 1375—1384.

Sheppard, H., Rodegker, W. 1962 — Determination of ^{3}H and ^{14}C in biological materials using oxygen bomb combustion — Analyt. Biochem. 4: 246—251.

Table I

Radioactivity of the organic horizons of the soil profile expressed as counts per second/mg dry weight (significance refers to comparison with background using Student's test)

Depth (cm)	Activity (c.p.s./mg)		
	after 2 months	after 6 months	after 20 months
0—1	26.1	21.4	9.3
1—2	not significant	0.89	1.48
2—3	not significant	not significant	0.19
below 3	—	—	not significant

Table II

Radioactivity of some arthropods from 20 months samples

Species	Number of individuals counted	Dry weight/sample (mg)	c.p.s./mg
Platynothrus peltifer (C.L.K.)	50	2.71	7.51
Adoristes poppei (Ouds.)	50	1.12	5.99
Ceratoppia bipilis (Herm.)	100	3.34	2.81
Steganacarus magnus (Nic.)	42	4.96	4.46
Phthiracarus sp.	21	1.61	3.92
Pergamasus-Eugamasus spp.	49	4.31	0.91
Collembola (undetermined)	not counted	2.70	2.85
Millipede	1	10.00	0.67